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Molecular Detection of Resistance and Virulence Genes in Coagulase Negative Staphylococci Isolated from Blood Cultures at the University Teaching Hospital of Bouake

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Abstract

Introduction: Coagulase-negative staphylococci (CoNS) are currently recognized as genuine pathogens. However, little is known about the resistance and virulence genes that explain their pathogenicity in hospitals in Côte d'Ivoire. The aim of this study was to contribute to the genotypic identification of resistance and virulence genes in CoNS isolated from blood cultures at the University Teaching Hospital (CHU) of Bouaké, in order to improve patient management. **Material and Methods:** This was a descriptive study conducted from September to December 2023. The CoNS isolates studied came from the collection of strains isolated from blood cultures of febrile patients hospitalized or attending consultations at the CHU of Bouaké. The strains were analyzed using conventional simplex PCR. **Results**: Of the 45 isolates analyzed, 46.7% carried both the *aacA-aphD* and *tetK* genes *and* 40% carried the *mecA* gene. With regard to virulence genes, only the *LukS-PV* gene was observed in *S. epidermidis and S. haemolyticus* isolates. **Conclusion:** The high prevalence of CoNS isolates carrying the *mecA* gene and the presence of virulence genes observed in this study give cause for concern in hospitals. It is important to develop comprehensive surveillance strategies against nosocomial and multi-resistant infections at the CHU of Bouaké.

Keywords

Coagulase-Negative Staphylococcus, Gene, Multiresistance, Virulence, Bouaké

1. Introduction

Staphylococci are bacteria of the genus *Staphylococcus* that can cause ubiquitous infections, constituting a major public health problem in various countries around the world [1]. The natural reservoirs of staphylococci are humans, warm-blooded animals and the environment [2]. Depending on their ability to produce an enzyme called coagulase, Staphylococci are divided into Coagulase Positive and Coagulase Negative (CoNS). According to many authors, CoNS, long considered contaminants, are now recognized as genuine pathogens [2] [3] [4]. In particular, their pathogenic power is well established in bacteremia, in immuno-depressed patients following surgery or the insertion of intra-vascular devices (bone or cardiac prostheses, probes, catheters, etc). Vascular catheters are the main entry point for these bacteremias [1] [5] [6] [7]. The morbidity associated with nosocomial CoNS bacteremia results in prolonged hospital stays [8] and an attributable mortality rate that varies from 10 to 30% depending on the study [9]. This high morbidity and prolonged hospital stay suggest the existence of virulence factors linked to the secretion of numerous toxic and enzymatic substances and resistance to antibiotic molecules [10]. According to the WHO, antibiotic resistance is one of the most serious threats to global health, food security and socio-economic development today. It is a natural phenomenon, but the misuse of antibiotics in humans and animals accelerates the process, leading to longer hospital stays, higher medical expenses and increased mortality [11]. In current practice, the identification of CoNS species is not carried out systematically in most bacteriology laboratories. The identification of Staphylococci is focused on *Staphylococcus aureus*. However, given the significant pathogenic role of CoNS, there are no data on the phenotypic and genotypic characteristics of the CoNS strains circulating at the University Teaching Hospital (CHU) of Bouaké. It seemed appropriate to initiate this study in order to address this concern. The aim of this study is to contribute to the phenotypic and molecular identification of resistance and virulence genes in CoNS isolated from blood cultures at CHU Bouake in order to improve patient management.

2. Material and methods

2.1. Type, Period and Origin of Strains

This was a descriptive cross-sectional study conducted from September to December 2023. The CoNS isolates studied came from the collection of strains isolated from the blood cultures of febrile patients hospitalized or referred at the University Teaching Hospital of Bouake for bacteriological analysis. These strains were stored in cryotubes containing heart-brain broth supplemented with 15% glycerol at −80˚C. The 45 CoNS strains were phenotyped during routine analyses: *Staphylococcus xylosus* (n = 13); *Staphylococcus haemolyticus* (n = 12)*; Staphylococcus saprophyticus* (n = 5); *Staphylococcus lentus* (n = 5)*; Sta-*

phylococcus epidermidis (n = 4)*; Staphylococcus capitis* (n = 3); *Staphylococcus chromogenes* (n = 2)*; Staphylococcus sciuri* (n = 1). Sociodemographic data and clinical information were collected from patients' microbiological records.

2.2. Microbiological Analyses

2.2.1. Subculturing of CoNS Isolates

The CoNS isolates were subcultured onto non-selective and selective agar plates, and incubated at 37˚C for 18 to 24 hours. The culture media used were Columbia agar (Bio-Rad, Marmes-la Coquette, France) enriched with 5% fresh sheep blood, Chapman agar (Bio-Rad, Marmes-la Coquette, France) and nutrient agar. Bacterial isolates were first grown on Columbia agar medium enriched with 5% fresh sheep blood and incubated at 37˚C for 24 hours. The strains were then cultured on Chapman medium and nutrient agar. The reference strains used for the microbiological analysis were cefoxitin-susceptible *S. aureus* ATCC 29213 and cefoxitin-resistant *S. aureus* ATCC 43300 *S. aureus* ATCC 43300 resistant to cefoxitin. Bacteriological analyses were carried out at the molecular biology laboratory of the CHU Bouaké.

2.2.2. Genomic DNA Extraction

Genomic DNA extraction was performed on the 45 bacterial strains grown on nutrient agar. Depending on the density of the culture on the agar, 10 colonies of each bacterial culture were picked and placed in Eppendorf tubes containing l ml of sterile distilled water. The contents were centrifuged for 10 minutes at 4˚C at maximum speed (13,000 g). The supernatant was discarded and the pellets recovered. Then 300 µl of sterile distilled water was added and placed in a water bath at 100˚C for 10 min. Heat shock was performed by placing the extracts in a −80˚C freezer for 5 minutes. Finally, the extracts were centrifuged for 10 min at maximum speed. The supernatants were aliquoted into 1.5 ml Eppendorf tubes and stored at −20˚C and then at +4˚C for conventional PCR.

2.2.3. PCR Detection of Resistance and Virulence Genes

Conventional simplex PCR using specific primers for each targeted gene (**Table 1**) carried out molecular detection of resistance genes (*mecA*, *aacA-aphD*, *tetK* and *tetM*) and virulence genes (*LukS-PV* and *tst*). PCR amplification was performed in a 25 µl reaction mixture containing 3 µl of DNA extract, 6.25 µl of 2× GoTaq® G2 Hot Start Colorless Master Mix (Promega, Madison, USA), 1.25 µL of each forward and reverse primer (10 µM) and 13.25 µL of nuclease-free water. Amplification of all genes was performed on a thermal cycler (Applied Biosystems, Inc., CA). Cycle parameters for resistance gene amplification were as follows: initial denaturation at 94˚C for 3 min followed by 30 cycles of amplification at 94˚C for 30 s, annealing at 55˚C for 30 s and extension at 72˚C for 30s (except for the final cycle which was 4 min for the extension step) [12]. For the *LukS-PV* and *tst* genes, amplification was carried out according to the following pro-

gramme: initial denaturation at 95˚C for 5 min followed by 40 cycles of amplification at 95° for 3 s, annealing at 55˚C for 30 s and extension at 72˚C for 1 min and a final cycle with extension at 75˚C for 7 min [13] [14]. PCR products were analysed on a 1.5% agarose gel at 120 V for 50 min in 1 × TBE containing GelRed® 10,000 × nucleic acid dye using a 100 bp DNA ladder (Promega, USA) as a size marker. The DNA fragments were visualised under UV (ultraviolet) in a FastGen/Blue Green Transilluminator DE.

3. Results

3.1. Clinical Socio-Demographic Characteristics of Patients

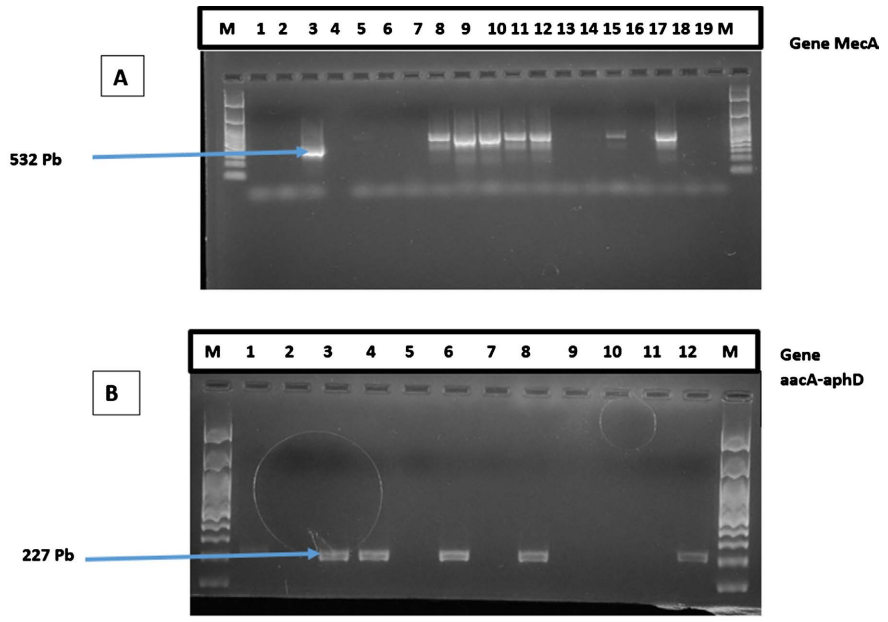
Analysis of the patients' socio-demographic characteristics showed that male patients were the most represented in 57.8% of cases, with a sex ratio of 1.37. The age groups most at risk were 0 - 15 years and 31 - 65 years, with 48.90% and 33.30% respectively. The clinical diagnosis of the patients revealed a suspicion of bacteremia with a body temperature greater than or equal to 39˚C. Patients were seen in consultations in eight clinical departments at CHU Bouake.

Table 1. Primers used for PCR.

Target gene	Primer pair	Primer sequence (5'-3')	Fragment size (pb)	Annealing at Temperature ˚C
Resistance gene				
MecA	*mecA1*	AAAATCGATGGTAAAGGTTGGC	532	55
	mecA2	AGTTCTGCAGTACCGGATTTGC		
Tetk	*tetk1*	GTAGCGACAATAGGTAATAGT	360	
	tetk2	GTAGTGACAATAAACCTCCTA		
TetM	*tetM1*	AGTGGAGCGATTACAGAA	158	
	tetM2	CATATGTCCTGGCGTGTCTA		
aacA-aphD	*aacA-aphD1*	TAATCCAAGAGCAATAAGGGC	227	
	aacA-aphD2	GCCACACTATCATAACCACTA		
Virulence gene				
LukS-PV	*LukS-PV*	GGCCTTTCCAATACAATATTGG	433	55
	LukF-PV	CCCAATCAACTTCATAAATTG		
Tst	*tst-1F*	GAAATTTTTCATCGTAAGCCCTTTGTTG	180	
	tst-1R	TTCATCAATATTTATAGGTGGTTTTTCA		

3.2. Genotypic Profile of CoNS Observed in Patients and Clinical Departments

Resistance genes (mecA, *aacA-aphD*, *tetK* and *tetM*) and virulence genes (*LuKS-PV*, *tst*) were searched for in all the different CoNS strains (Figure 1). PCR data showed that the strains harboured 46.7% of the *aacA-aphD* genes and 40% of the *mecA* gene. The *tetK* and *tetM* genes were found in 46.7% and 8.89% of the strains analyzed respectively. In terms of virulence genes, only the *LukS-PV* gene was observed in all strains, in 6.7% of cases. More specifically, the *mecA* gene was present in all strains with the exception of the *S. sciuri* strain. Among the *S. xylosus* strains (n = 13), 7 strains possessed the mecA resistance gene (53.8%), followed by *S. epidermidis* and *S. chromogenes* species with 50% each. The five *S. saprophyticus* strains identified in the study all harboured the *aacA-aphD* gene (100%), followed by *S. epidermidis* and *S. chromogenes* with 75 and 50% respectively. The highest percentages of *TetM* genes were found in *S. epidermidis* (25%) and S. *lentus* (20%). Half of the S. *epidermidis* strains isolated carried the *LukS-PV* virulence gene. The *tst* gene was not detected among the CoNS species analyzed (Table 2). The 0-15 and 31-65 age groups had the highest percentage of resistance and virulence genes. The External, Internal Medicine and Paediatrics departments recorded the highest rate of resistance genes. The *Luks-PV* virulence gene was found in the Neurosurgery, Neurology and Paediatrics departments (Table 3).



Part A-M: DNA marker (100 - 1500 pb); positive control: well 3; negative control: wells 1 and 2. PCR amplification of the MecA gene showing a single band at 532 pb (wells 8, 9, 10, 11, 12, 15, 17: MecA positive strains; wells 4, 5, 6, 7, 13, 14, 18: MecA negative strains). Part B-M: DNA marker (100 - 1500 pb); Positive control: well 12; Negative control: well 1. PCR amplification of the *aacA-aphD* gene showing a single band at 227 pb (Well, 3, 4, 6, 8: *aacA-aphD* positive strains; well 2; 5, 9, 11, *aacA-aphD* negative strains).

Figure 1. Electrophoresis of 1.5% agarose gel of CoNS *MecA and aacA-aphD* gene.

Table 2. Resistance and virulence genes observed in different CoNS species.

	Resistance gene				Virulence gene
	MecA Gene	*aacA-aphD Gene*	*TetK Gene*	*tetM Gene*	*LukS-PV Gene*
Species identified	n%	n%	n%	n%	n%
S. capitis (n = 3)	1 (33.3)	1 (33.3)	2 (66.70)	0	0
S. chromogenes (n = 2)	1 (50)	1 (50)	1 (50)	0	0
S. epidermidis (n = 4)	2 (50)	3 (75)	1 (25)	1 (25)	2 (50)
S. haemolyticus (n = 12)	4 (33.30)	4 (33.3)	6 (50)	1 (8.30)	1 (8.30)
S. lentus (n = 5)	1 (20)	1 (20)	1 (20)	1 (20)	0
S. saprophyticus (n = 5)	2 (40)	5 (100)	4 (80)	0	0
S. sciuri (n = 1)	0	0	1	0	0
S. xylosus (n = 13)	7 (53.80)	6 (46.1)	5 (38.50)	1 (7.70)	0

Table 3. Genes searched for according to age group and clinical services.

	Resistance Gene				Virulence Gene
	mecA	*aacA-aphD*	*TetK*	*tetM*	*LukSPV*
Age group	n%	n%	n%	n%	n%
0 - 15 ans	11 (61.1)	12 (57.1)	10 (47.6)	2 (50)	1 (33.3)
16 - 25 ans	1 (5.6)	1 (4.7)	1 (4.8)	0	0
26 - 30 ans	0	0	0	0	0
31 - 65 ans	5 (27.8)	8 (38)	9 (42.8)	2 (50)	1 (33.3)
≥66 ans	1 (5.6)	0	1 (4.8)	0	1 (33.3)
Clinical services					
External (n = 4)	4 (100)	2 (50)	3 (75)	0	0
Internal medicine (n = 10)	4 (40)	4 (40)	4 (100)	0	0
Neurosurgery (n = 2)	1 (50)	2 (100)	2 (100)	0	1 (50)
Neurology (n = 2)	0	1 (50)	0	1 (50)	1 (50)
Paediatrics (n = 18)	7 (38.9)	10 (55.6)	7 (38.9)	2 (11.1)	1 (5.6)
Pneumo-Phthisiology (n = 1)	0	0	1 (100)	0	0
Intensive care (n = 4)	1 (25)	2 (50)	3 (75)	0	0
Medical emergencies (n = 4)	1 (25)	0	1 (25)	1 (25)	0

4. Discussion

Coagulase-negative staphylococci (CoNS) were previously considered contaminants of microbiology laboratories or pathogens of low virulence. Despite their low virulence, they have invasive and destructive potential, as observed in certain cases of endocarditis on natural valves [15]. The virulence characteristics of these strains are not well understood. Some authors have reported that the pathogenesis of CoNS in patients is linked to the insertion of medical devices such as catheters, central venous lines, urinary catheters and cardiac valve prostheses, which cause septicaemia, bacteraemia, endocarditis and urinary tract infections [16] [17]. The aim was to contribute to the molecular identification of virulence and resistance genes in CoNS strains isolated from blood cultures, in order to improve patient management. The CoNS were isolated from blood cultures from patients seen in consultation or hospitalization at the CHU Bouaké. Analysis of the epidemiological and clinical data of the patients from whom the strains were isolated showed that 57.80% of the patients were male, with a sex ratio of 1.37:1. The age group most at risk was 0-15 years in 48.9% of cases. The frequency of isolation in clinical departments was 40% in paediatric and 22.2% in internal medicine. This result corroborates that of [18], in which the frequency of isolation of CoNS in blood cultures was higher in paediatric wards (47.2%), followed by medical wards (44.1%). This predominance of CoN*S* in the aforementioned department could be explained by the high demand for bacteriological analysis due to awareness raising by the Bacteriology department among paediatricians, and by the existence of other risk factors such as catheterisation of peripheral venous lines and the fragile immune system of newborns, as supported by [19] [20]. These results are similar to those of Gbonon in his study of the ecology and antibiotic susceptibility of bacteria isolated from maternal-foetal infections, which noted a frequency of 65.38% of CoNS from positive blood cultures [9]. The present study provides the first report from CHU Bouaké on the frequency of the *mecA* gene encoding PLP2a. PLP2a, which has a low affinity for betalactam antibiotics, particularly meticillin, is not affected by these antibiotics and thus allows the bacteria to continue biosynthesising its cell wall [21]. The *aacA-aphD* gene of the *Staphylococcus* Tn4001 transposon is a determinant of resistance to Aminosides [22]. This gene specifies resistance to gentamicin, tobramycin and kanamycin. It was sought in isolates with the KTG resistance phenotype. Two main mechanisms of tetracycline resistance have been described in *S. aureus*: active efflux, resulting from the acquisition of the *tetK* and *tetL* genes located in the plasmid, and ribosomal protection by elongation factor-type proteins encoded by chromosomal *tetM* or *tetO* [23]. In this study, the primers *tetK* and *tetM* were used to search for tetracycline resistance genes in isolates. Virulence-related genes encoding Panton-Valentine leukocidin toxin (*Luk-PV*) and the *tst* gene encoding staphylococcal toxic shock toxin (*tst*) were also identified in the various isolates. From a total of 45 CoNS isolates, 18 (40%) carried the *mecA* gene. Other resistance genes such as *aacA-aphD and tetM* were found in

46.7% and 8.89% respectively. As for virulence genes, only the *LukS-PV* gene was observed in 6.7% of isolates. In light of these results, there is a discrepancy between the phenotypic detection of methicillin resistance using cefoxitin screening and the absence of the *mecA* gene in CoNS strains. This may be attributed to the fact that methicillin resistance is caused by mechanisms other than mecA gene expression [24]. Furthermore, the sensitivity of PCR in the detection of mecA may have been compromised by the presence of PCR inhibitors or other physical factors [25] [26]. Furthermore, it was observed in this study that *mecA*-negative isolates resistant to cefoxitin have *mecA* alleles that could not be detected by the primers used in this study. This is because many CoNS strains also exhibit diversity in *mecA* sequences and have a different impact on *β*-lactam resistance [25]. The results of work by Mohammad *et al.* showed that out of 15 isolates with a phenotype resistant to oxacillin or cefoxitin, only one possessed the *mecA* gene [27]. It has been noted that there are unusual methicillin-resistant CONSs that have a resistance mechanism other than PBP2a production and these have been reported as borderline methicillin-resistant strains [28]. Methicillin-resistant borderline strains are resistant to oxacillin due to their plasmid determinants, including hyperproduced penicillinases, genes conferring resistance to cadmium or other gene products [29]. It is also possible that cefoxitin-resistant *mecA*-negative CoNS have mecA alleles that could not be detected by the primers used in this study. Many CoNS strains also show diversity in mecA sequences and have a different impact on *β*-lactam resistance. The prevalence of the different genes according to CoNS species showed that *S. xylosus* species harboured 53.8% of the *mecA* gene. However, the *aacA-aphD* gene was present in all the *S. saprophyticus* species isolated and identified, and 50% of *S. epidermidis* species possessed the *LukS-PV* virulence gene, followed by 25% with the *TetM gene.* The *LukS-PV* virulence gene was most frequently observed in patients aged 0 - 15 years. The presence of multi-drug resistant CoNS in the hospital setting, presenting resistance and then virulence genes in patients of all ages and more particularly in those aged 0 - 15 years, is a major concern for clinicians. And this situation may be the main cause of failure in the treatment of infectious diseases, increased morbidity and mortality and the evolution of new pathogens.

5. Conclusion

The study of CoNS is becoming increasingly relevant, both clinically and epidemiologically, as evidenced by the number of infections, morbidity, and mortality attributed to them. Although most of them are classified as simple contaminants, they can in some cases be the cause of infections. There is a marked diversity of CoNS species at Bouaké University Hospital. The CoNS are therefore a crucial aetiological agent of human infections, particularly in paediatric and intensive care units where the use of medical devices is common. This study is one of the first to demonstrate a high incidence of CoNS in blood cultures in Bouaké, based

on resistance and virulence genes. A high prevalence of strains carrying the mecA gene was reported in the CoNs analyzed. The presence of virulence and resistance genes observed in this study on CoNS is becoming a cause for concern in the hospital environment. It is of the utmost importance to develop comprehensive surveillance and prevention strategies that will lead to effective and robust control not only of CoNS but also of other multi-resistant infections. Key elements of these strategies include the implementation of active screening procedures tailored to the specific endemic situation, adherence to basic infection control practices, the introduction and monitoring of micro-organism-specific hygiene measures, and antibiotic control programs. The increasing availability of new-generation sequencing tools due to their falling cost has not yet been reflected in Côte d'Ivoire, by their limited use in routine investigations, at least in the large microbiology laboratories. The acquisition of benchtop high-throughput sequencing equipment will be important for investigating outbreaks of CoNS infections with reduced turnaround times. This will lead to a thorough assessment of the true infectious/virulent potential of isolated CoNS species and inform rapid clinical decisions.

Current State of Knowledge on the Subject

- The CoNS, long considered contaminants, are now recognized as genuine pathogens;
- The frequency of isolation of CoNS in blood cultures is higher in paediatrics;
- The high morbidity and prolonged hospital stay suggest the existence of virulence factors linked to the secretion of numerous toxic and enzymatic substances and resistance to antibiotic molecules in CoNS.

Contribution of Our Study to Knowledge

- Almost half of the CoNS isolates contained the aacA-aphD, TetK and mecA genes;
- The presence of CoNS virulence and resistance genes is becoming a cause for concern in hospitals, and surveillance and prevention strategies need to be introduced against CoNS and other multi-resistant bacteria.

Authors' Contributions

All authors have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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